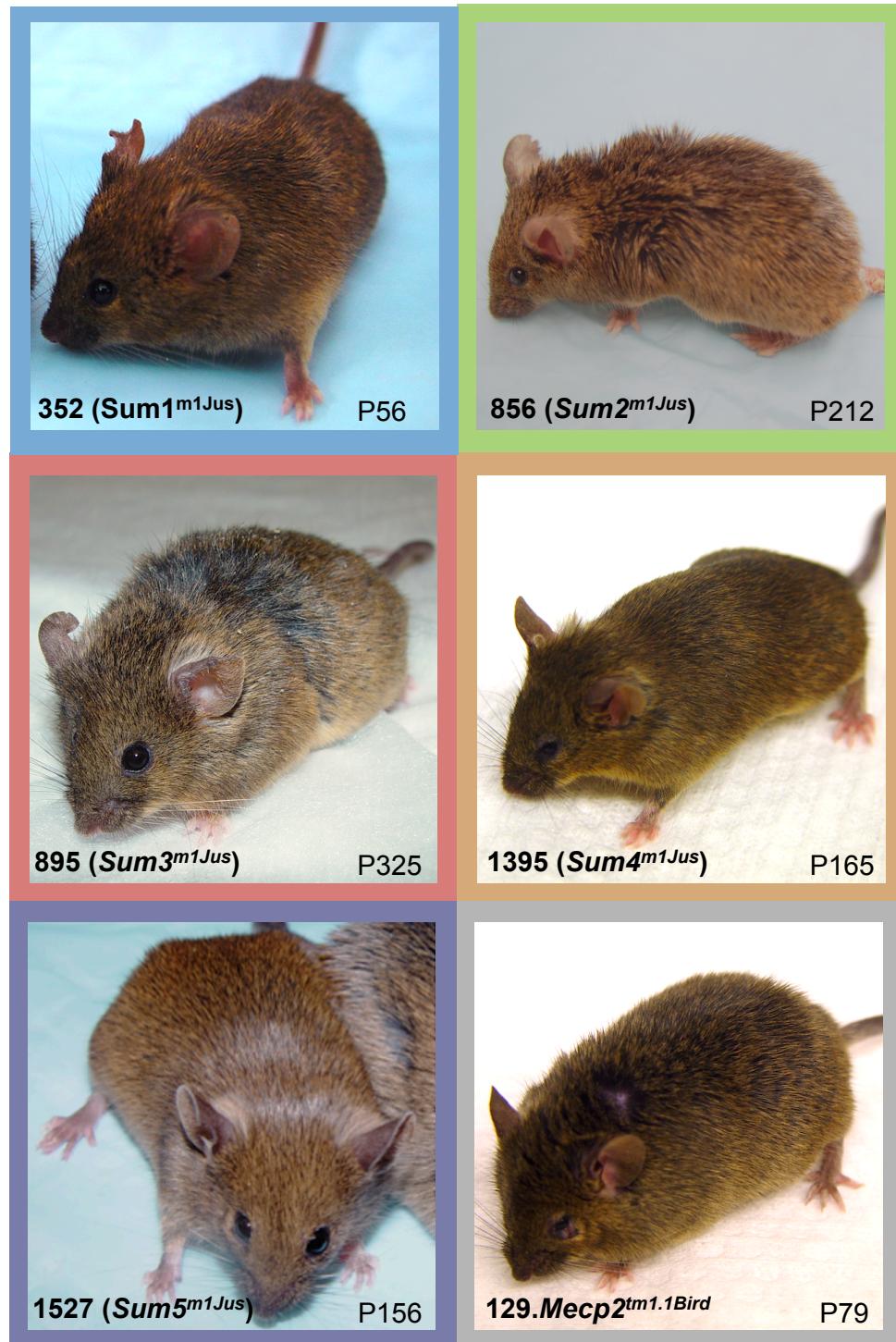


Supplementary Material

A suppressor screen in mouse *Mecp2* implicates cholesterol metabolism in Rett Syndrome

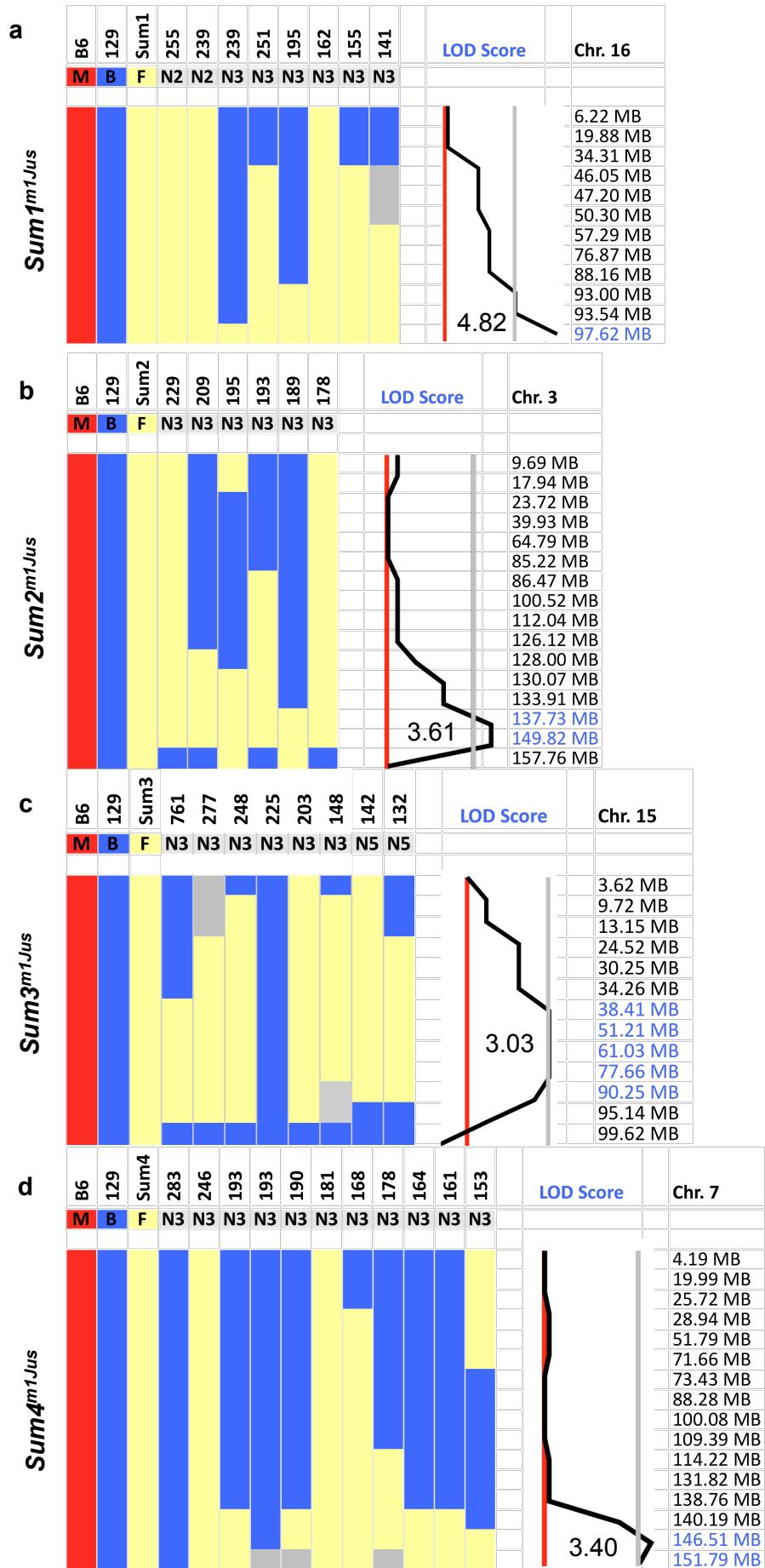
Christie M. Buchovecky, Stephen D. Turley, Hannah M. Brown, Stephanie M. Kyle, Jeffrey G. McDonald, Benny Liu, Andrew A. Pieper, Wenhui Huang, David M. Katz, David W. Russell, Jay Shendure, and Monica J. Justice

Supplementary Figure 1: Founder animals from each suppressor line and 129.*Mecp2*^{tm1.1Bird}/Y shown at postnatal day (P). Mice carrying *Sum* mutations have increased longevity and appear significantly healthier longer.

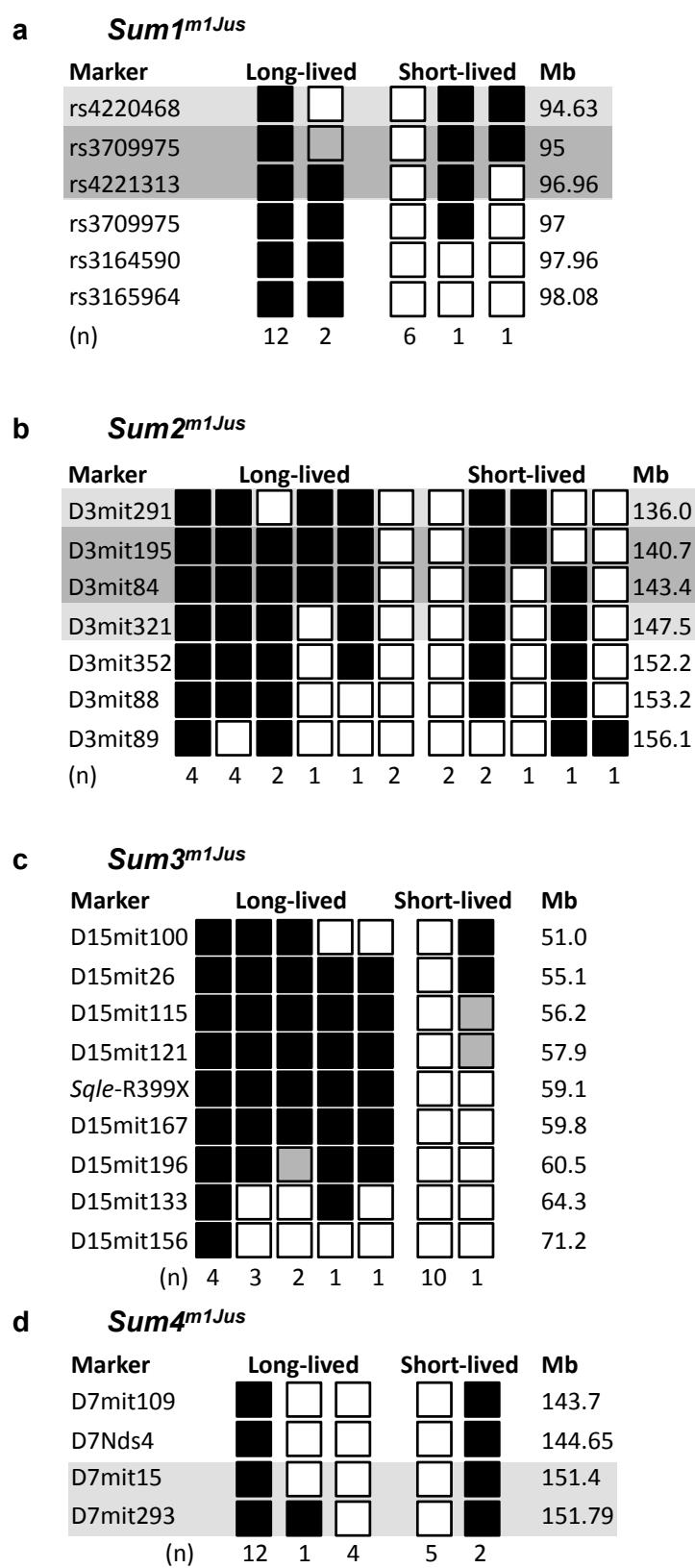


Supplementary Figure 2:

Map locations for four modifiers were identified. Data from informative SNPs are shown for each founder line (F). The mutated strain (M) is in red; background strain (B) blue; heterozygotes yellow; and missing calls grey. The vertical red line indicates a LOD score of zero, and the grey line a LOD of 3. Longevity in days is shown above the plot and megabase (Mb) position is shown to the right (only long-lived animals are shown). **a)** *Sum1^{m1Jus}* maps to chromosome 16, LOD score = 4.82. **b)** *Sum2^{m1Jus}* maps to chromosome 3, LOD score = 3.61. **c)** *Sum3^{m1Jus}* maps to chromosome 15, LOD score = 3.03. **d)** *Sum4^{m1Jus}* maps to chromosome 7, LOD score = 3.40. The maximum LOD score for *Sum5^{m1Jus}* was < 3.0, and a map location could not be confirmed.

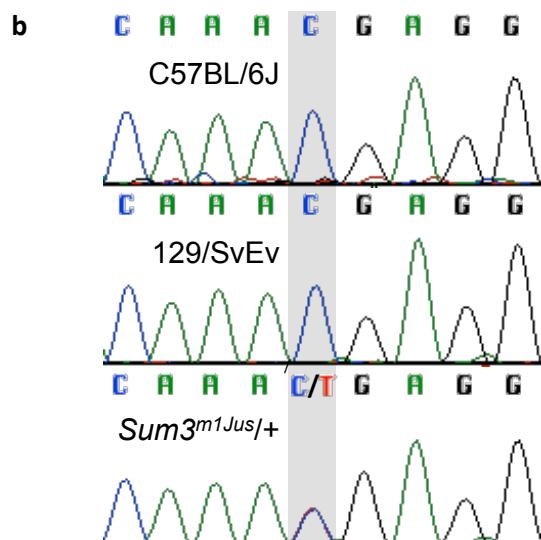


Supplementary Figure 3: Map
locations were refined for all four
modifiers using additional SNPs or
MIT markers. Data from fine
mapping analysis are shown for **a)**
Sum1^{m1Jus}, **b)** *Sum2^{m1Jus}*, **c)**
Sum3^{m1Jus}, and **d)** *Sum4^{m1Jus}*.
Each locus of homozygous 129S6/
SvEvTac background strain DNA
is depicted by a white box. The
Sum locus for each line maps to a
heterozygous region, depicted by
black boxes. Any missing calls are
depicted by a grey box. The
megabase (Mb) position is shown
to the right. The number (n) of
long-lived or short-lived animals
with a particular genotype are
displayed at the bottom of each
column. Highlighted rectangles
represent putative map locations.



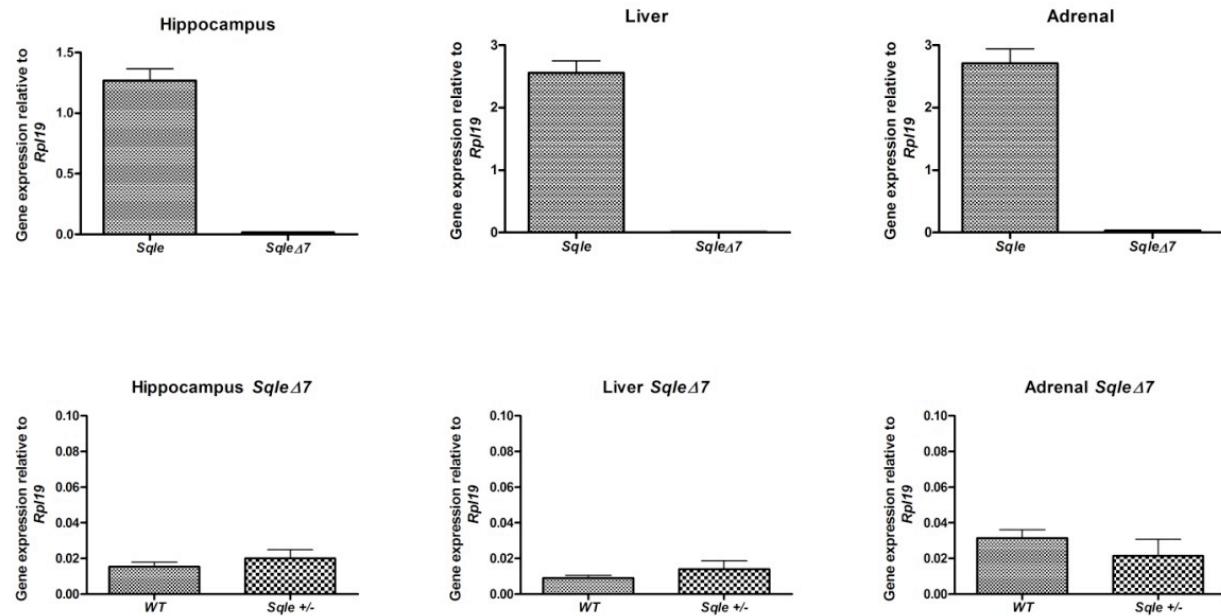
Supplementary Figure 4: Mapping results were combined with whole exome sequencing data to identify the *Sum3^{m1Jus}* lesion. **a)** Heterozygous non-synonymous or splice-site variants were identified by whole exome sequencing. Only one lesion occurred in both mutant animals (#N₃-64 and #N₃-206), but not in controls, and fell within the line 895 map location. **b)** Sanger sequencing confirmed the ENU-induced *Sq/e* R399X mutation.

a	#64	#206	both
Nonsynonymous mutations/splice variants	1688	1432	1081
...not observed in control mouse exomes	328	172	7
...intersecting with <i>Sum3^{m1Jus}</i> map location	3	4	1



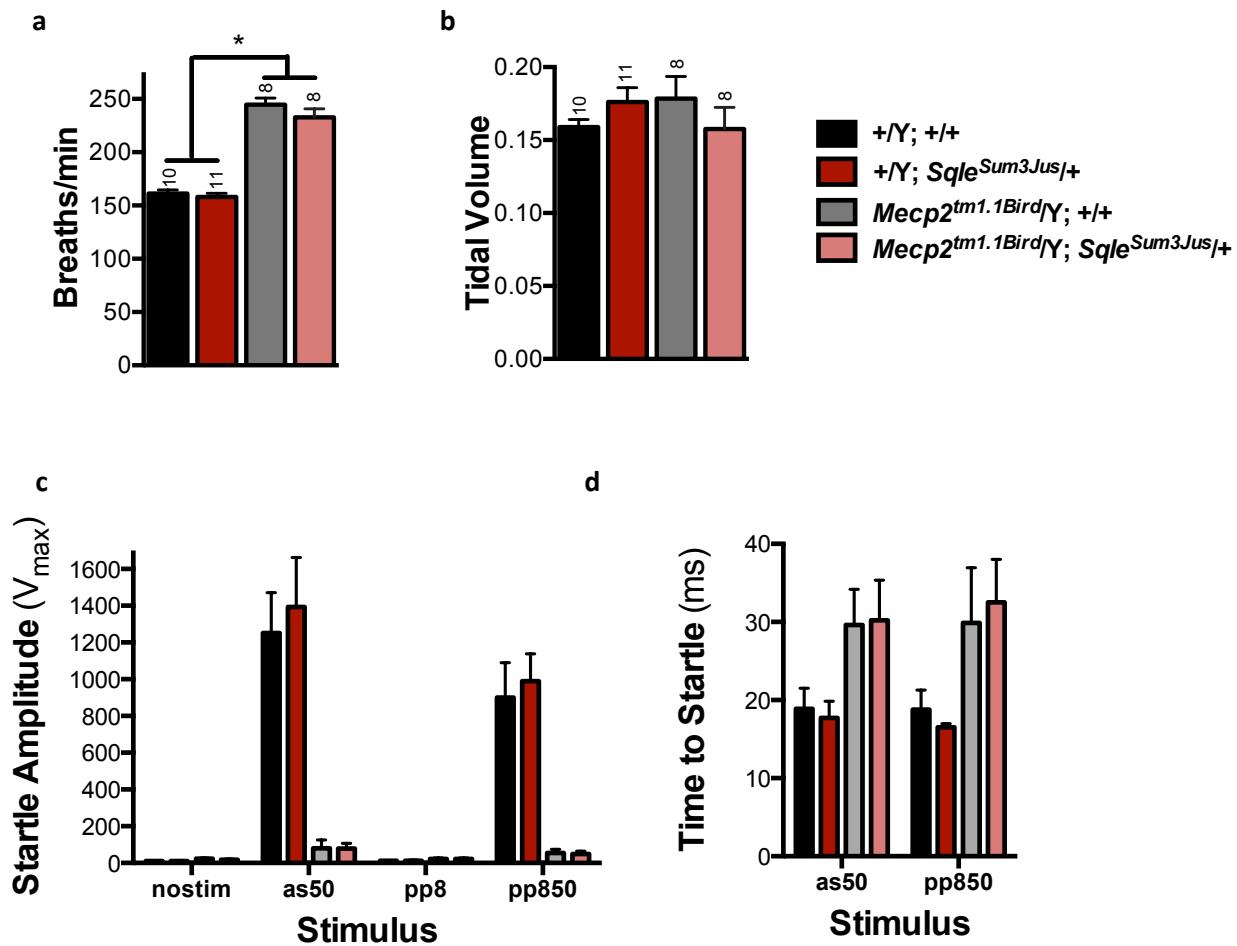
Supplementary Figure 5: Expression of *Sqle* isoforms in mouse tissues.

Three mice of each genotype were analyzed at 10 weeks of age. All error bars represent s.e.m.

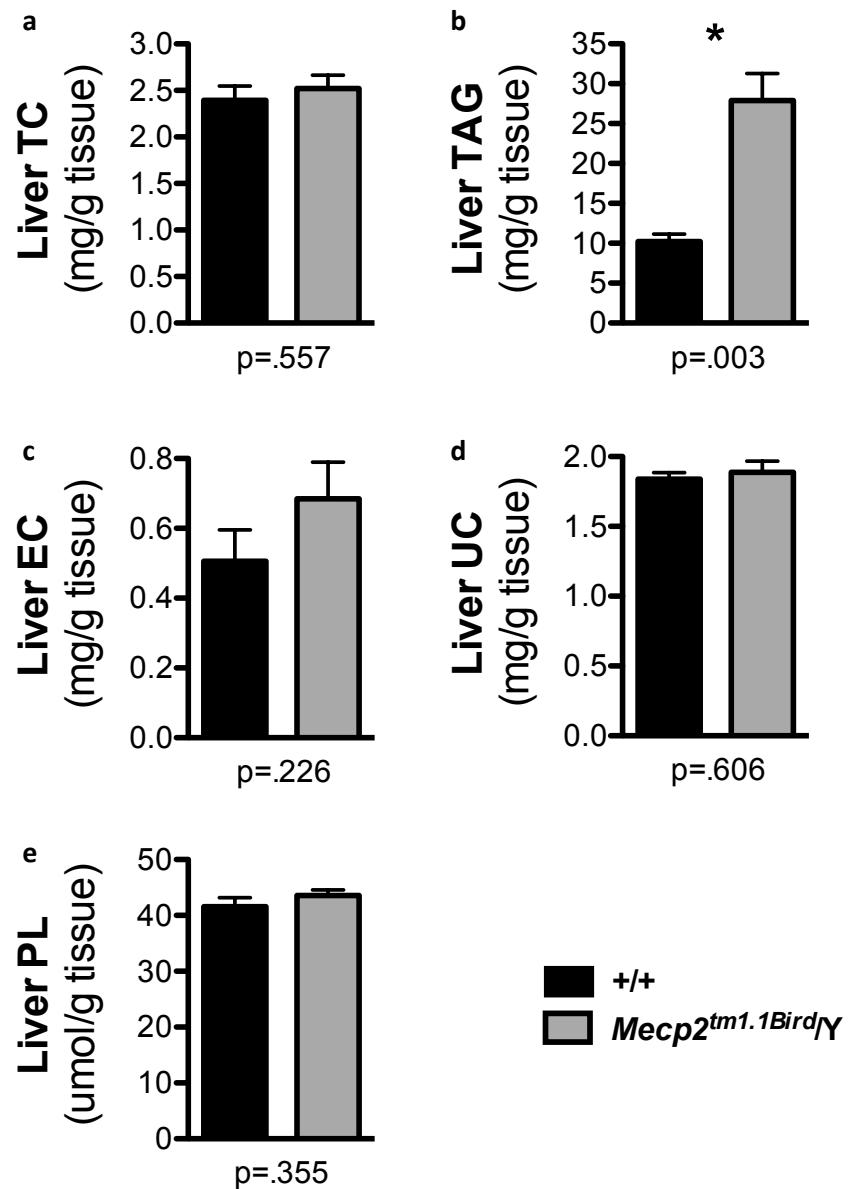


Supplementary Figure 6: Additional phenotyping data for *Mecp2*^{tm1.1Bird/Y}; *Sqle*^{Sum3Jus/+} mice.

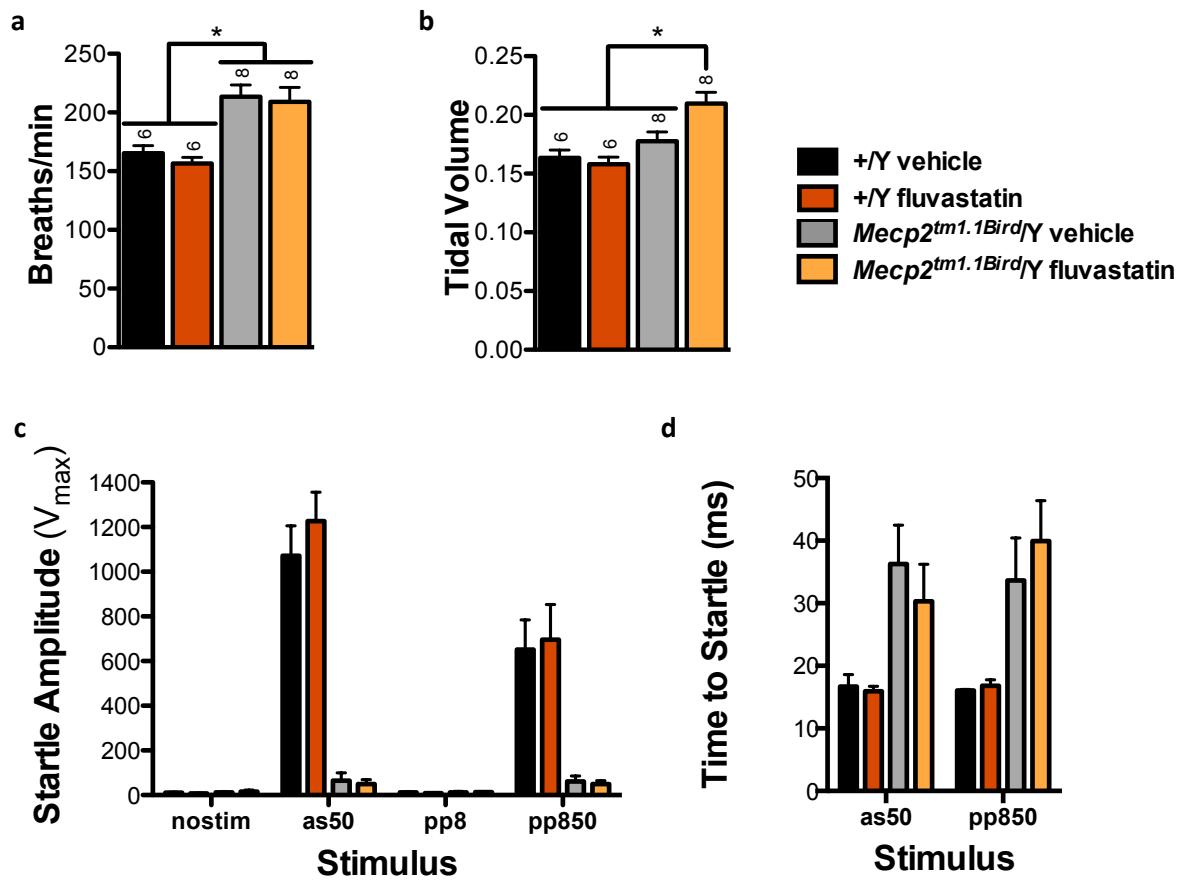
Unrestrained whole-body plethysmography at P70 shows that **a)** the number of breaths per minute is significantly higher in all *Mecp2*^{tm1.1Bird/Y} mice, regardless of *Sqle*^{Sum3Jus} mutation status ($p < .05$) and **b)** tidal volume is not significantly changed between groups. Furthermore, the *Sqle*^{Sum3Jus} mutation does not **c)** increase startle amplitude nor **d)** decrease time to startle in 129.*Mecp2*^{tm1.1Bird/Y} mice that undergo a pre-pulse inhibition assay at P70. nostim=No stimulus presented; as50=50dB stimulus presented; pp8=8dB pre-pulse presented; pp850=8dB pre-pulse followed by 50dB stimulus. All error bars represent s.e.m.



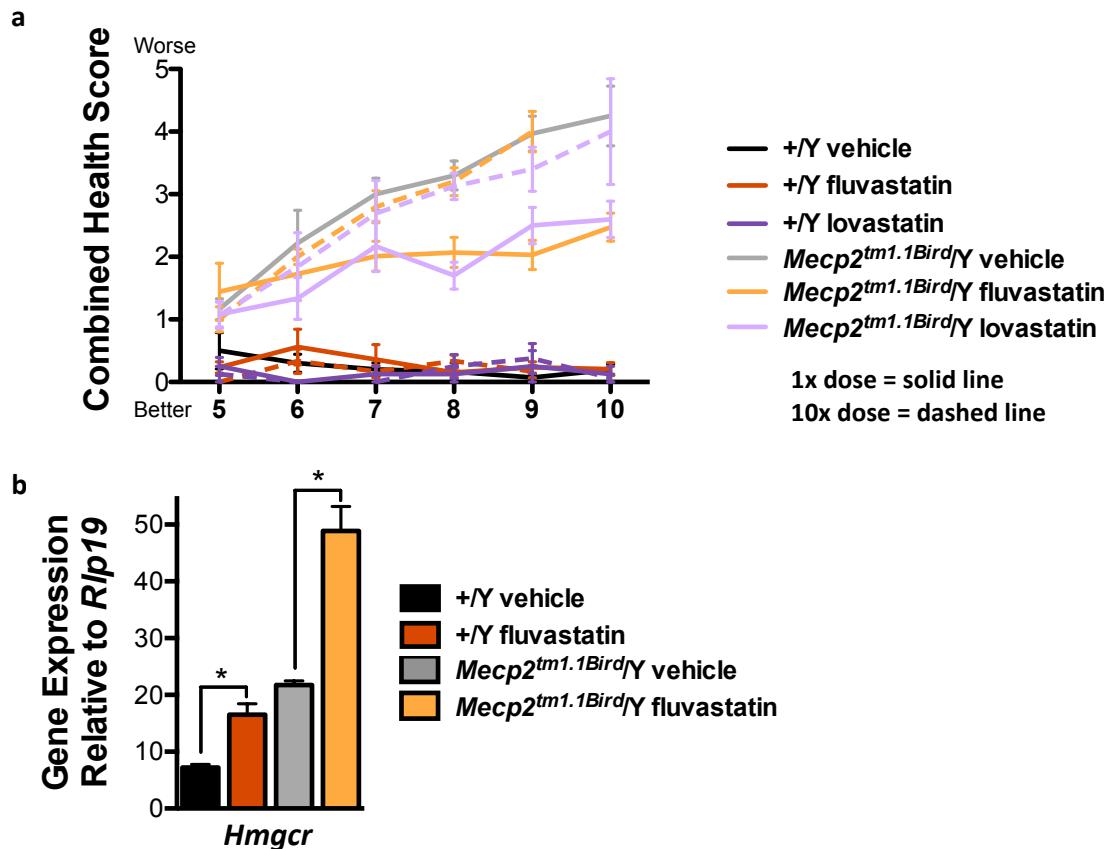
Supplementary Figure 7: Triglyceride buildup is the primary contributor to fatty liver in 129.*Mecp2*^{tm1.1Bird}/Y mice. Liver lipid analysis was performed on 129.*Mecp2*^{tm1.1Bird}/Y mice and wild type littermates at P56 (N=6 per genotype). The concentration of **a)** total cholesterol (TC) was unchanged while **b)** triglycerides (TAG) were elevated in 129.*Mecp2*^{tm1.1Bird}/Y mice. **c,d)** Total cholesterol can be subdivided into esterified and unesterified cholesterol (EC and UC, respectively). **e)** The concentration of total phospholipids (PL) was also unchanged. All error bars represent s.e.m.



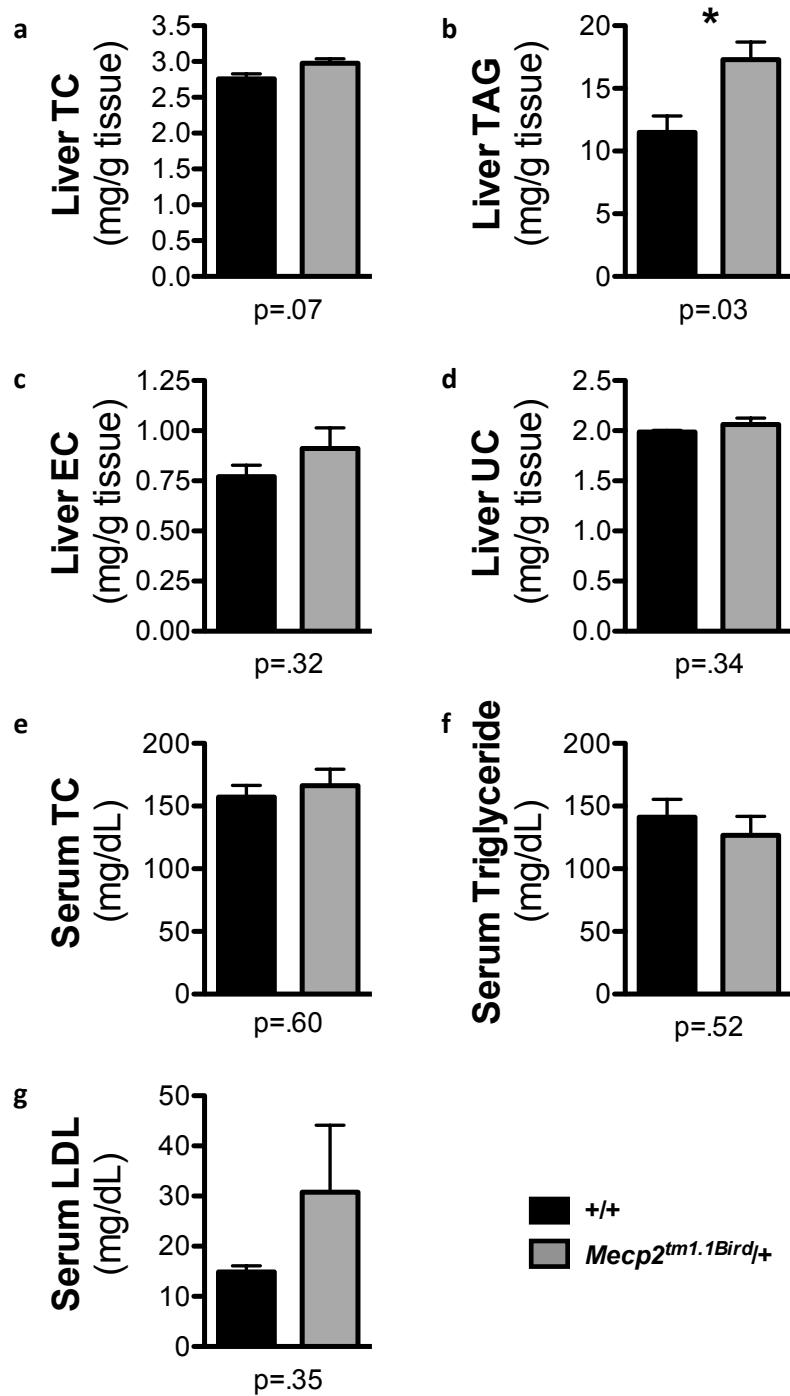
Supplementary Figure 8: Additional phenotyping data for fluvastatin treated *Mecp2*^{tm1.1Bird/Y} mice. Unrestrained whole-body plethysmography at P56 shows that **a)** the number of breaths per minute is significantly higher in all *Mecp2*^{tm1.1Bird/Y} mice, regardless of fluvastatin treatment ($p < .05$) and **b)** tidal volume is not significantly changed between groups. Furthermore, fluvastatin treatment does not **c)** increase startle amplitude nor **d)** decrease time to startle in 129.*Mecp2*^{tm1.1Bird/Y} mice that undergo a pre-pulse inhibition assay at P70. nostim=No stimulus presented; as50=50dB stimulus presented; pp8=8dB pre-pulse presented; pp850=8dB pre-pulse followed by 50dB stimulus. All error bars represent s.e.m.



Supplementary Figure 9: Cohorts of 129.*Mecp2*^{tm1.1Bird}/Y and +/Y mice were treated with multiple doses of statins: vehicle (0 mg/kg), fluvastatin (low = 3 mg/kg, high = 30 mg/kg), lovastatin (low = 1.2 mg/kg, high = 12 mg/kg). **a)** Subjective health scores show that 129.*Mecp2*^{tm1.1Bird}/Y mice treated with a high (10x) statin dose do not perform as well as the lower dose. Maximum subjective health score is 6, a combined score of 0(better)-2(worse) for limbclasping, tremors, and activity. Mice were scored using the assessment published in Guy, et al (2007), where a score of 0 is not different from wildtype, 1 is more severe and 2 is very severe. **b)** Fluvastatin treatment (3 mg/kg dose) produces a compensatory increase in *Hmgcr* expression in the liver, regardless of *Mecp2* genotype. Expression data shown is for liver tissue collected 24 hours after the last injection at P70. All error bars represent s.e.m.



Supplementary Figure 10: Triglyceride buildup in *Mecp2^{tm1.1Bird/+}* liver precedes serum dyslipidemia. Liver lipid analysis and serum lipid panels were performed on *Mecp2^{tm1.1Bird/+}* mice and wild type littermates at P56 (N=4 per genotype). In the liver, the concentration of **a)** total cholesterol (TC) was unchanged while **b)** triglycerides (TAG) were elevated in *Mecp2^{tm1.1Bird/+}* mice. **c,d)** Total cholesterol can be subdivided into esterified and unesterified cholesterol (EC and UC, respectively). Both of these are unchanged in *Mecp2^{tm1.1Bird/+}* mice. In the serum, **e)** total cholesterol, **f)** triglyceride and **g)** LDL cholesterol were unchanged. The elevated levels of triglycerides stored in the liver represent the earliest signs of peripheral dyslipidemia. All error bars represent s.e.m.



Supplementary Table 1: Breeding data from heterozygous *Sqle*^{Sum3Jus} parents.

Total Litters:	14
Average litter # at birth	3.93
Average litter # at weaning	3.86
Average perinatal loss	0.07

Parents: *Sqle*^{Sum3Jus}/+ x *Sqle*^{Sum3Jus}/+

Pup genotype:	+/+	<i>Sqle</i> ^{Sum3Jus} /+	<i>Sqle</i> ^{Sum3Jus} / <i>Sqle</i> ^{Sum3Jus}
Expected Ratio (%)	25	50	25
Expected #	13.5	27	13.5
Actual Ratio (%)	39	61	0
Actual #	21	33	0

Litter	Pups born	Pups weaned	<i>Sum3</i> WT	<i>Sum3</i> +/-	<i>Sum3</i> -/-
1	9	8	3	6	0
2	5	5	1	4	0
3	2	2	1	1	0
4	4	4	1	3	0
5	5	5	2	3	0
6	4	4	3	1	0
7	1	1	0	1	0
8	4	4	4	0	0
9	3	3	0	3	0
10	3	3	2	1	0
11	3	3	0	3	0
12	4	4	1	3	0
13	5	5	2	3	0
14	3	3	1	2	0
Total:	55	54	21	34	0

Supplementary Table 2: Primers used for RT-PCR analysis of cholesterol biosynthesis.

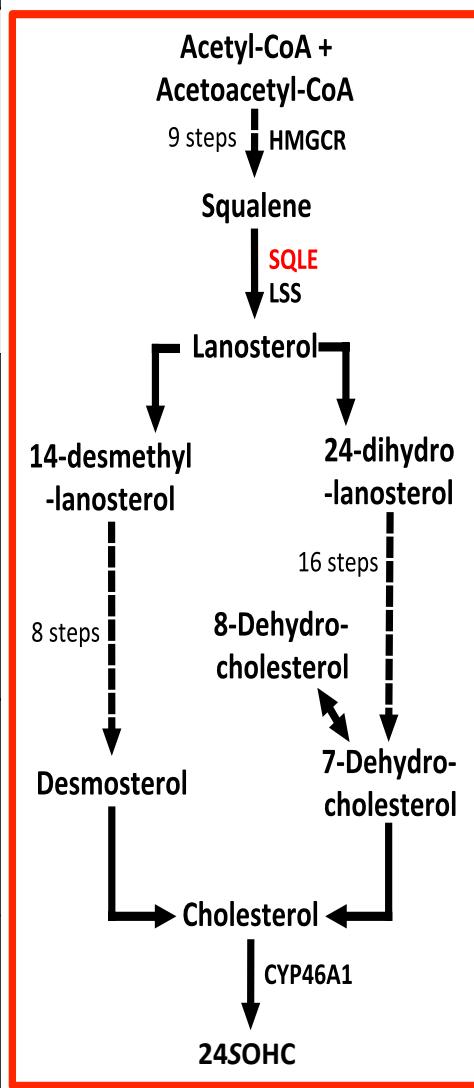
Gene	Forward (5'-3')	Reverse (5'-3')	product (bp)
<i>Rpl19</i>	tcccgagttacagcaccttgac	cacggcttggcttcatttaac	110
<i>Cyp46a1</i>	tccctccgttgcgcacct	ggccatgacaacttcacct	190
<i>Sqle</i>	cagcttccttccttccttcct	tggaaaatagcagcatcatca	239
<i>Hmgcr</i>	tgtggccagcactaacagag	ggcctcttatcactgcaa	178

Supplementary Table 3a: P70 individual data from Tandem Mass Spectrometry analysis for cholesterol precursors lanosterol (LAN), desmosterol (DES), 8-dehydrocholesterol (8-DHC) and 7-dehydrocholesterol (7-DHC) in 129.*Mecp2*^{tm1.1Bird}/Y mice and their wildtype littermates. Expressed as ng/mg.

Sample	Genotype		Drug Treatment	Tissue	Age	LAN	DES	8-DHC	7-DHC
	<i>Mecp2</i>	<i>Sqle</i>							
P70-WTV1	WT	WT	vehicle	Brain	P70	4.50	128.02	9.08	2.94
P70-WTV2	WT	WT	vehicle	Brain	P70	3.63	121.80	8.09	2.76
P70-WTV3	WT	WT	vehicle	Brain	P70	5.61	158.18	12.61	3.09
P70-WTV4	WT	WT	vehicle	Brain	P70	3.90	125.94	8.95	2.41
P70-WTF1	WT	WT	fluv	Brain	P70	4.47	126.93	10.93	2.65
P70-WTF2	WT	WT	fluv	Brain	P70	3.62	125.75	8.39	2.54
P70-WTF3	WT	WT	fluv	Brain	P70	3.62	134.22	8.90	2.35
P70-WTF4	WT	WT	fluv	Brain	P70	3.67	153.02	11.20	2.41
P70-NV1	null/Y	WT	vehicle	Brain	P70	2.22	86.59	8.17	2.30
P70-NV2	null/Y	WT	vehicle	Brain	P70	2.36	89.01	6.48	2.48
P70-NV3	null/Y	WT	vehicle	Brain	P70	1.66	94.86	11.26	3.71
P70-NV4	null/Y	WT	vehicle	Brain	P70	2.69	97.82	7.05	2.20
P70-NF1	null/Y	WT	fluv	Brain	P70	3.85	95.16	11.27	4.02
P70-NF2	null/Y	WT	fluv	Brain	P70	2.52	102.21	8.84	3.19
P70-NF3	null/Y	WT	fluv	Brain	P70	2.29	117.15	10.01	3.24
P70-NF4	null/Y	WT	fluv	Brain	P70	2.45	100.78	8.37	2.97
P70-WT1	WT	WT	--	Brain	P70	3.49	98.97	11.65	3.26
P70-WT2	WT	WT	--	Brain	P70	5.71	109.38	13.90	3.04
P70-WT3	WT	WT	--	Brain	P70	4.70	114.89	14.02	3.40
P70-WT4	WT	WT	--	Brain	P70	4.92	120.32	15.02	3.80
P70-WT5	WT	WT	--	Brain	P70	4.43	129.50	12.08	4.18
P70-WT6	WT	WT	--	Brain	P70	4.78	134.46	15.19	3.23
P70-WTS1	WT	Mutant	--	Brain	P70	3.36	83.06	11.17	4.22
P70-WTS2	WT	Mutant	--	Brain	P70	3.15	84.77	11.71	3.78
P70-WTS3	WT	Mutant	--	Brain	P70	3.58	92.37	13.98	4.61
P70-WTS4	WT	Mutant	--	Brain	P70	3.59	99.33	14.60	3.84
P70-WTS5	WT	Mutant	--	Brain	P70	3.68	100.80	12.40	3.95
P70-N1	null/Y	WT	--	Brain	P70	3.18	76.40	12.09	2.20
P70-N2	null/Y	WT	--	Brain	P70	3.16	90.66	11.45	3.93
P70-N3	null/Y	WT	--	Brain	P70	3.04	92.57	13.95	4.45
P70-N4	null/Y	WT	--	Brain	P70	2.70	95.21	14.49	3.40
P70-N5	null/Y	WT	--	Brain	P70	3.07	109.55	14.38	4.65
P70-N6	null/Y	WT	--	Brain	P70	2.76	110.08	11.44	5.57
P70-NS1	null/Y	Mutant	--	Brain	P70	1.56	53.30	7.55	3.65
P70-NS2	null/Y	Mutant	--	Brain	P70	2.26	67.05	9.60	4.42
P70-NS3	null/Y	Mutant	--	Brain	P70	2.01	73.79	11.27	4.22
P70-NS4	null/Y	Mutant	--	Brain	P70	1.97	74.60	11.31	4.75
P70-NS5	null/Y	Mutant	--	Brain	P70	2.91	76.51	11.53	3.06
P70-NS6	null/Y	Mutant	--	Brain	P70	2.25	83.09	11.68	5.52

Supplementary Table 3b: P56 individual data from Tandem Mass Spectrometry analysis for cholesterol precursors lanosterol (LAN), desmosterol (DES), 8-dehydrocholesterol (8-DHC) and 7-dehydrocholesterol (7-DHC) in 129.Mecp2^{tm1.1Bird}/Y mice and their wildtype littermates. Expressed as ng/mg.

Sample	Genotype		Tissue	Age	LAN	DES	8-DHC	7-DHC
	<i>Mecp2</i>	<i>Sqle</i>						
P56-WT1	WT	WT	Brain	P56	3.54	94.56	9.90	3.58
P56-WT2	WT	WT	Brain	P56	5.60	129.66	14.22	3.56
P56-WT3	WT	WT	Brain	P56	3.48	111.74	12.00	3.44
P56-WT4	WT	WT	Brain	P56	5.24	96.48	12.60	3.26
P56-WT5	WT	WT	Brain	P56	4.02	93.96	11.90	3.08
P56-WT6	WT	WT	Brain	P56	6.12	145.89	16.96	2.65
P56-WT7	WT	WT	Brain	P56	5.31	139.20	14.46	2.83
P56-WT8	WT	WT	Brain	P56	5.29	137.90	18.89	3.00
P56-N1	null/Y	WT	Brain	P56	3.10	68.00	11.58	3.18
P56-N2	null/Y	WT	Brain	P56	3.08	86.00	8.46	1.76
P56-N3	null/Y	WT	Brain	P56	5.60	101.62	8.46	2.28
P56-N4	null/Y	WT	Brain	P56	1.82	90.86	11.18	2.46
P56-N5	null/Y	WT	Brain	P56	4.55	101.69	14.82	3.74
P56-N6	null/Y	WT	Brain	P56	6.24	101.57	17.19	7.47
P56-N7	null/Y	WT	Brain	P56	3.89	98.00	15.65	4.69
P56-N8	null/Y	WT	Brain	P56	4.54	93.30	13.62	4.45
P56-WT1	WT	WT	Liver	P56	0.96	1.14	1.52	2.78
P56-WT2	WT	WT	Liver	P56	1.20	1.50	1.42	1.52
P56-WT3	WT	WT	Liver	P56	0.58	1.72	1.86	1.30
P56-WT4	WT	WT	Liver	P56	0.82	1.24	1.22	1.26
P56-WT5	WT	WT	Liver	P56	1.00	1.24	1.00	1.66
P56-N1	null/Y	WT	Liver	P56	1.38	1.20	0.50	0.80
P56-N2	null/Y	WT	Liver	P56	4.84	1.88	2.46	1.62
P56-N3	null/Y	WT	Liver	P56	1.60	1.42	1.52	0.96
P56-N4	null/Y	WT	Liver	P56	0.96	1.78	3.94	1.84



Supplementary Table 4a: Individual data for cholesterol synthesis measurements *in vivo*. Measurements taken in B6.*Mecp2*^{tm1.1Jae}/Y mice and their wildtype littermates at P60.

Mouse #	Genotype	Body Weight g	Organ Weight						
			Liver	Spleen	Kidney	Lung	Brain	Testis	Carcass
1	+/Y	24.1	1.42	0.067	0.337	0.14	0.438	0.145	20.047
3	+/Y	23.9	1.377	0.068	0.303	0.14	0.437	0.151	19.553
5	+/Y	22.7	1.271	0.072	0.298	0.131	0.427	0.167	18.411
7	+/Y	21.7	1.299	0.065	0.283	0.124	0.406	0.161	17.844
2	<i>Mecp2</i> /Y	21.8	1.172	0.056	0.304	0.117	0.371	0.146	17.813
4	<i>Mecp2</i> /Y	24.2	1.461	0.057	0.29	0.108	0.381	0.145	19.804
6	<i>Mecp2</i> /Y	25	1.392	0.05	0.317	0.12	0.379	0.149	20.347
8	<i>Mecp2</i> /Y	25.8	1.366	0.043	0.289	0.113	0.373	0.14	21.131
9	<i>Mecp2</i> /Y	19.4	1.122	0.057	0.242	0.125	0.353	0.126	15.493

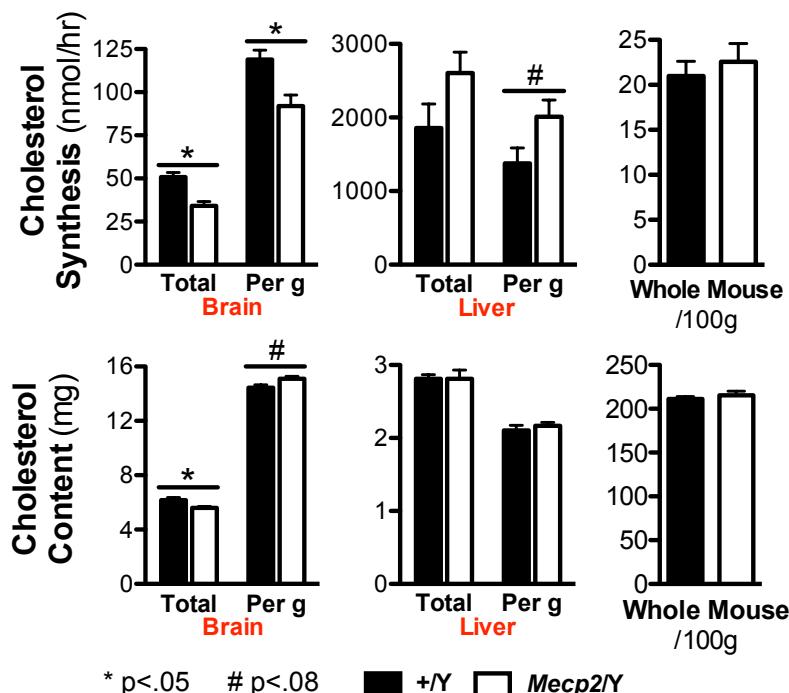
Mouse #	Genotype	Plasma Water SA cpm/nmol	Tissue Cholesterol Synthesis						
			Liver	Spleen	Kidney	Lung	Brain	Testis	Carcass
1	+/Y	31	1795	281.5	284.9	328.3	125.9	171.1	148.7
3	+/Y	30.59	1446	269.8	292.2	339	107	133.8	150.2
5	+/Y	32.18	759	157	259.8	184.8	130.3	123.3	138.4
7	+/Y	30.9	1476	275.4	272.2	327.1	112.4	98.3	151.1
2	<i>Mecp2</i> /Y	33.95	2656	375.5	278.3	410.3	97.3	92	168.8
4	<i>Mecp2</i> /Y	37.34	1459	264.9	372.9	311.3	107.5	105	102
6	<i>Mecp2</i> /Y	35.22	2411	344	300.1	389	88.4	87	129.6
8	<i>Mecp2</i> /Y	39.82	1885	360.2	272.8	335.4	69.3	78	97.9
9	<i>Mecp2</i> /Y	35.4	1645	271.3	192.1	240.9	97.2	109	151

Mouse #	Genotype	Cholesterol Synthesis umol/hr/100g bw	Whole Organ Cholesterol Synthesis						
			Liver	Spleen	Kidney	Lung	Brain	Testis	Carcass
1	+/Y	23.94	2548.9	18.9	96	46	55.1	24.8	2981
3	+/Y	21.54	1991.1	18.4	88.6	47.5	46.8	20.2	2936.3
5	+/Y	16.31	964.7	11.3	77.4	24.2	55.6	20.6	2548.9
7	+/Y	22.17	1917.3	17.9	77	40.6	45.6	15.8	2697
2	<i>Mecp2</i> /Y	29.01	3112.8	21	84.6	48	36.1	13.5	3007.2
4	<i>Mecp2</i> /Y	18.03	2131.6	15.1	108.2	33.6	41	15.2	2019
6	<i>Mecp2</i> /Y	24.79	3356.1	17.2	95.1	46.7	33.5	12.9	2637.2
8	<i>Mecp2</i> /Y	18.76	2574.9	15.5	78.8	37.9	25.8	10.9	2067.9
9	<i>Mecp2</i> /Y	22.29	1845.7	15.5	46.5	30.1	34.3	13.7	2338.8

Supplementary Table 4b: Individual data for plasma and tissue cholesterol levels in organs of animals used for cholesterol synthesis experiments. Measurements taken in B6.*Mecp2*^{tm1.1Jae}/Y mice and their wildtype littermates at P60. All error bars represent s.e.m.

Mouse #	Genotype	Serum Cholesterol Concentration mg/dl	Tissue Total Cholesterol Concentration mg/g					
			Liver	Spleen	Kidney	Lung	Brain	Testis
1	+/Y	86	1.96	3.06	4.04	5.08	14.78	2.51
3	+/Y	81.2	2.09	2.97	3.82	4.98	14.88	2.47
5	+/Y	86.6	2.3	2.3	4.23	5.04	14.08	2.71
7	+/Y	79.8	2.06	2.06	4.09	4.52	14	2.44
2	<i>Mecp2</i> /Y	71.4	2.17	2.99	4.21	4.85	15.25	2.77
4	<i>Mecp2</i> /Y	111.4	2.12	3.53	4.07	5.3	14.72	2.61
6	<i>Mecp2</i> /Y	88.9	2.22	4.12	3.85	4.9	14.83	2.58
8	<i>Mecp2</i> /Y	94.6	2.02	2.93	4.17	5.06	15.71	2.72
9	<i>Mecp2</i> /Y	84.7	2.3	2.9	4.35	4.32	14.97	2.51
								2.26

Mouse #	Genotype	Whole Animal Cholesterol Content mg/100g bw	Whole Organ Cholesterol Content mg/organ					
			Liver	Spleen	Kidney	Lung	Brain	Testis
1	+/Y	202.9	2.78	0.2	1.36	0.71	6.47	0.36
3	+/Y	210.88	2.88	0.2	1.16	0.7	6.5	0.37
5	+/Y	216.43	2.92	0.22	1.26	0.66	6.01	0.45
7	+/Y	214.33	2.67	0	1.16	0.56	5.68	0.39
2	<i>Mecp2</i> /Y	220.32	2.54	0.17	1.28	0.57	5.66	0.4
4	<i>Mecp2</i> /Y	207.64	3.1	0.2	1.18	0.57	5.61	0.38
6	<i>Mecp2</i> /Y	211.28	3.09	0.21	1.22	0.59	5.62	0.38
8	<i>Mecp2</i> /Y	205.66	2.75	0.13	1.2	0.57	5.85	0.38
9	<i>Mecp2</i> /Y	231.91	2.58	0.17	1.05	0.54	5.28	0.32
								35.05



Supplementary Table 5: Injection and phenotyping schedules for drug studies.

Fluvastatin + Vehicle Treated ♂ ♂	Age	Event	Fluvastatin + Vehicle Treated ♂♂	Age	Event
P42	Injection		P35	Injection	
P49	Injection		P42	Injection	
P56	Injection		P49	Injection	
P63	Injection		P54	Injection	
P70	Injection		P56	Injection	
P77	Injection		P57	Rotarod	
P84	Injection		P58	Rotarod, Injection	
P91	Injection		P61	Injection	
P98	Injection		P63	Injection	
P105	Injection		P65	Injection	
P112	Injection		P68	Injection	
P119	Injection		P69	Open Field, Pre-Pulse Inhibition	
P126	Injection		P70	Injection	
P133	Injection		P71	Plethysmography, Tissue Collection	
P134	Open Field				
P140	Injection				
P141	Rotarod				
P142	Rotarod				
P147	Injection				
P154	Injection				
P161	Injection				
P168	Injection				
P175	Injection				
P182	Injection				
P189	Injection				
P196	Injection				
P203	Injection				
P210	Injection				
P217	Injection				
P224	Injection				
P231	Injection				
P232	Tissue Collection				

Lovastatin + Vehicle Treated ♂♂	Age	Event
P34	Injection	
P37	Injection	
P41	Injection	
P44	Injection	
P48	Injection	
P51	Injection	
P55	Injection	
P56	Rotarod	
P57	Rotarod	
P58	Injection	
P62	Injection	
P65	Injection	
P69	Injection	
P70	Open Field	
P71	Plethysmography, Tissue Collection	